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(71) Applicant: WISCONSIN ALUMNI RESEARCH FOUNDATION
614 North Walnut Street
Madison, WI 53705(US)

(72) Inventor: DeLuca, Hector F.
1809 Highway BB Deerfield
Wisconsin 53531(US)
Inventor: Schnoes, Heinrich K.
1806 Summit Avenue
Madison Wisconsin 53705(US)
Inventor: Perlman, Kato L.
1 Chippewa Court
Madison Wisconsin 53711(US)
Inventor: Sicinski, Rafal R.
University of Warsaw Dept. of Chemistry
02-093 Warsaw Pasteura 1(PL)
Inventor: Prahl, Jean Martin
7 Georgetown Court
Madison Wisconsin 53919(US)

(74) Representative: Ellis-Jones, Patrick George Armine et al
J.A. KEMP & CO. 14 South Square Gray's Inn
London WC1R 5LX(GB)

(54) 19-Nor vitamin D compounds.

(57) This invention provides a novel class of vitamin D-related compounds, namely the 1α-hydroxy-19-nor-vitamin D analogs, as well as a general method for their chemical synthesis. The compounds exhibit pronounced activity in arresting the proliferation of undifferentiated cells, including malignant cells, and in inducing their differentiation, and thus represent novel therapeutic agents for the treatment of malignant and other diseases characterized by the proliferative grointh of undifferentiated cells. Formulations for therapeutic use and treatment methods are also provided.

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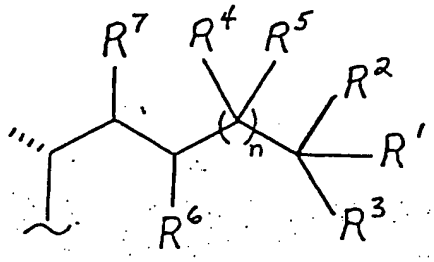
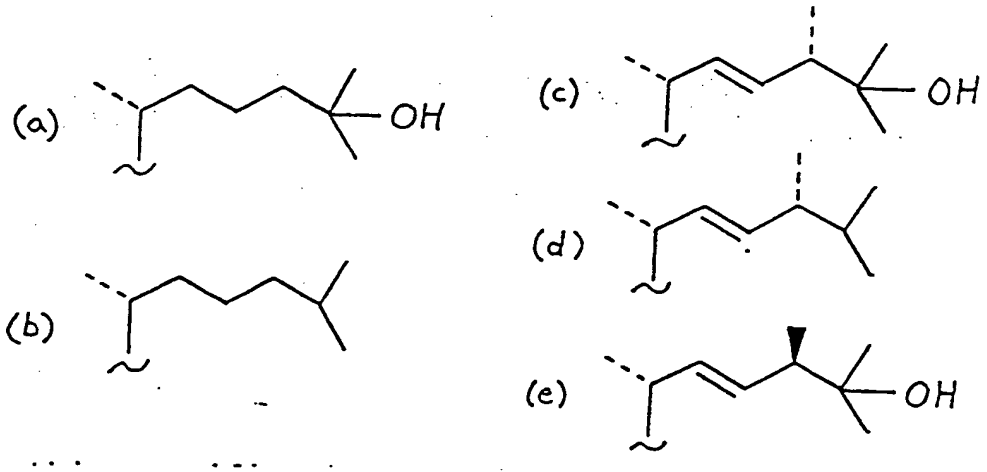


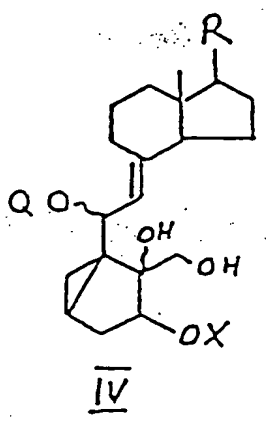
wherein R^1 represents hydrogen, hydroxy or O-acyl, R^2 and R^3 are each selected from the group consisting of alkyl, hydroxyalkyl and fluoroalkyl, or, when taken together represent the group -- $(CH_2)_m$ -- where m is an integer having a value of from 2 to 5, R^4 is selected from the group consisting of hydrogen, hydroxy, fluorine, O-acyl, alkyl, hydroxyalkyl and fluoroalkyl, R^5 is selected from the group consisting of hydrogen, fluorine, alkyl, hydroxyalkyl and fluoroalkyl, or, R^4 and R^5 taken together represent double-bonded oxygen, R^6 and R^7 are each selected from the group consisting of hydrogen, hydroxy, O-acyl, fluorine and alkyl, or, R^6 and R^7 taken together form a carbon-carbon double bond, and wherein n is an integer having a value of from 1 to 5, and wherein the carbon at any one of positions 20, 22, or 23 in the side chain may be replaced by an O, S, or N atom.

Specific important examples of side chains are the structures represented by formulas (a), (b), (c), (d) and (e) below, i.e. the side chain as it occurs in 25-hydroxyvitamin D_3 (a); vitamin D_3 (b); 25-hydroxyvitamin D_2 (c); vitamin D_2 (d); and the C-24-epimer of 25-hydroxyvitamin D_2 (e).

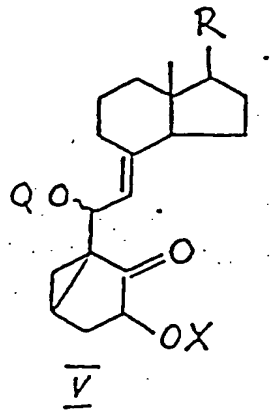


In this specification and the claims, the term 'alkyl' signifies an alkyl radical of 1 to 5 carbons in all isomeric forms, such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, etc., and the terms 'hydroxyalkyl' and 'fluoroalkyl' refer to such an alkyl radical substituted by one or more hydroxy or fluoro groups respectively, and the term 'acyl' means an aliphatic acyl group of 1 to 5 carbons, such as formyl, acetyl, propionyl, etc. or an aromatic acyl group such as benzoyl, nitrobenzoyl or halobenzoyl. The term 'aryl' signifies a phenyl-, or an alkyl-, nitro- or halo-substituted phenyl group.

The preparation of 1α-hydroxy-19-nor-vitamin D compounds having the basic structure shown above can be accomplished by a common general method, using known vitamin D compounds as starting materials. Suitable starting materials are, for example, the vitamin D compounds of the general structure II:



IV

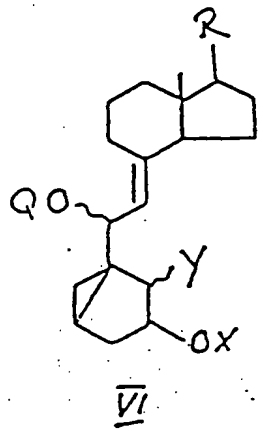


V

These two consecutive steps can be carried out according to the procedures given by Paaren et al. [J. Org. Chem. 48, 3819 (1983)]. If the side chain unit, R, carries vicinal diols (e.g. 24,25-dihydroxy or 25,26-dihydroxy, etc.), these, of course, also need to be protected, e.g. via acylation, silylation, or as the isopropylidene derivative prior to the periodate cleavage reactions.

In most cases, the acylation of the 1α-hydroxy group as mentioned above will simultaneously effect the acylation of side chain hydroxy functions, and these acylation conditions can, of course, be appropriately adjusted (e.g. elevated temperatures, longer reaction times) so as to assure complete protection of side chain vicinal diol groupings

The next step of the process comprises the reduction of the 10-oxo-group to the corresponding 10-alcohol having the structure VI shown below (where X is acyl and Y represents hydroxy). When X is acyl, this reduction is carried out conveniently in an organic solvent at from, say, 0°C to room temperature, using $NaBH_4$ or equivalent hydride reducing agents, selective for the reduction of carbonyl groups without cleaving ester functions. Obviously, when X is a hydroxy-protecting group that is stable to reducing agents, any of the other hydride reducing agents (e.g. $LiAlH_4$, or analogous reagents) may be employed also.



VI

The 10-hydroxy intermediate can then be treated with an alkyl- or arylsulfonylhalide (e.g. methanesulfonyl-chloride) in a suitable solvent (e.g. pyridine) to obtain the corresponding 10-O-alkyl-or arylsulfonyl derivative (the compound having the structure shown VI above, where Y is alkyl-SO_2O-, or aryl-SO_2O-, and this sulfonate intermediate is then directly reduced, e.g. with lithiun aluminum hydride, or the analogous known lithium aluminum alkyl hydride reagents in an ether solvent, at a temperature typically from 0°C to the boiling temperature of the solvent, thereby displacing the sulfonate group and obtaining the 10-deoxy derivative, represented by the structure VI above, where X and Y are both hydrogen. As shown by the above structure, a 1-O-acyl function in the precursor compound V is also cleaved in this reduction step to produce the free 1α-hydroxy function, and any O-acyl protecting group in the side chain would, of course, likewise be reduced to the corresponding free alcohol function, as is well understood in the art. If desired, the hydroxy groups at C-1 (or hydroxy groups in the side chain) can be reprotected by acylation or

Table 1

Differentiation of HL-60 Cells			
1α,25-dihydroxyvitamin D_3	% Differentiated Cells		
(moles/liter)	(mean ± SEM)		
	NBT	NSE	PHAGO
1×10^{-7}	86 ± 2	89 ± 1	87 ± 3
1×10^{-8}	60 ± 2	60 ± 3	64 ± 2
1×10^{-9}	33 ± 2	31 ± 2	34 ± 1
1α,25-dihydroxy-19-nor-vitamin D_3, (Ia)			
(moles/liter)			
2×10^{-7}	94 ± 2	95 ± 3	94 ± 2
1×10^{-7}	90 ± 4	84 ± 4	90 ± 4
5×10^{-8}	72 ± 3	73 ± 3	74 ± 3
1×10^{-8}	61 ± 3	60 ± 3	56 ± 1
1×10^{-9}	32 ± 1	31 ± 1	33 ± 1

In contrast to the preceding results, the new 19-nor analog (Ia) exhibits no activity in an assay measuring the calcification of bone, a typical response elicited by vitamin D compounds. Relevant data, representing the results of an assay comparing the bone calcification activity in rats of 1α,25-dihydroxyvitamin D_3 and 1α,25-dihydroxy-19-nor-vitamin D_3 (Ia), are summarized in Table 2. This assay was conducted according to the procedure described by Tanaka et al., Endocrinology 92, 417 (1973).

The results presented in Table 2 show the expected bone calcification activity of 1α,25-dihydroxyvitamin D_3 as reflected by the increase in percent bone ash, and in total ash at all dose levels. In contrast, the 19-nor analog Ia exhibits no activity at all three dose levels, when compared to the vitamin D-deficient (-D) control group.

Table 2

Calcification Activity			
Compound	Amount Administered*	% Ash	Total Ash (mg)
	(pmoles/day/7 days)	(mean ± SEM)	(mean ± SEM)
-D (control)	0	19 ± 0.8	23 ± 1.2
1α,25-dihydroxy-vitamin D_3	32.5	23 ± 0.5	34 ± 1.6
	65.0	26 ± 0.7	36 ± 1.1
	325.0	28 ± 0.9	40 ± 1.9
1α,25-dihydroxy-19-nor-vitamin D_3 (Ia)	32.5	22 ± 0.9	28 ± 1.6
	65.0	19 ± 1.5	28 ± 3.4
	325.0	19 ± 1.2	30 ± 2.4

* Each assay group comprised 6 rats, receiving the indicated amount of test compound by intraperitoneal injection daily for a period of seven days.

Thus the new 19-nor analog shows a selective activity profile combining high potency in inducing the differentiation of malignant cells with very low or no bone calcification activity. The compounds of this novel structural class, therefore, can be useful as therapeutic agents for ihc treatment of malignancies. Because the differentiative activity of vitamin D compounds on keratinocytes of skin (Smith et al., J. Invest. Dermatol.

cyclovitamin D derivative (Va, X=Ac). Mass spectrum m/z (relative intensity) 442 (M^+-MeOH) (18), 424 (8), 382 (15), 364 (35), 253 (55), 225 (25), 197 (53), 155 (85), 137 (100). ^{1}H NMR ($CDCl_3$) δ 0.58 (3H, s, 18-CH_3), 0.93 (3H, d, J=6.6 Hz, 21-CH_3), 1.22 (6H, s, 26-CH_3 and 27-CH_3), 2.15 (s, 3-$OCOCH_3$), 3.30 (3H, s, 6-OCH_3), 4.61 (1H, d, J=9.1 Hz, 6-H), 4.71 (1H, d, J=9.6 Hz, 7-H), 5.18 (1H, m, 1β-H).

It has been bound also that this diol cleavage reaction does not require elevated temperatures, and it is, indeed, generally prefereable to conduct the reaction at approximately room temperature.

(d) 1α-Acetoxy-10.25-dihydroxy-3.5-cyclo-19-nor-vitamin D_3 6-methyl ether (VIa, X-Ac, Y=OH):

The 10-oxo derivative Va (X=Ac) (2.2 mg, 4.6 µmol) was dissolved in 0.5 ml of ethanol and to this solution 50 µl (5.3 µmol) of a $NaBH_4$ solution (prepared from 20 mg of $NaBH_4$, 4.5 ml water and 0.5 ml of 0.01 N NaOH solution) was added and the mixture stirred at 0°C for ca. 1.5 h, and then kept at 0°C for 16 h. To the mixture ether was added and the organic phase washed with brine, dried over $MgSO_4$, filtered and evaporated. The crude product was purified by column chromatography on a 15 x 1 cm silica gel column and the alcohol VIa (X=Ac, Y=OH) was eluted with ethyl acetate hexane mixtures to give 1.4 mg (3 µmol) of product. Mass spectrum m/z (relative intensity) 476 (M^+) (1), 444 (85), 426 (18), 384 (30), 366 (48), 351 (21), 255 (35), 237 (48), 199 (100), 139 (51), 59 (58).

(e) 1α.25-Dihydroxy-19-nor-vitamin D_3 (Ia, $X^1=X^2=H$):

The 10-alcohol (VIa, X=Ac, Y=OH) (1.4 mg) was dissolved in 100 µl anhydrous CH_2Cl_2 and 10 µl (14 µmol) triethylamine solution [prepared from 12 mg (16 µl) triethylamine in 100 µl anhydrous CH_2Cl_2], followed by 7 µl (5.6 µmol) mesyl chloride solution (9 mg mesyl chloride, 6.1 µl, in 100 µl anhydrous CH_2Cl_2) added at 0°C. The mixture was stirred at 0°C for 2 h. The solvents were removed with a stream of argon and the residue (comprising compound VIa, X=Ac, Y=CH_3SO_2O-) dissolved in 0.5 ml of anhydrous tetrahydrofuran; 5 mg of $LiAlH_4$ was, added at 0°C and the mixture kept at 0°C for 16h. Excess $LiAlH_4$ was decomposed with wet ether, the ether phase was washed with water and dried over $MgSO_4$, filtered and evaporated to give the 19-nor product VIa (X=Y=H).

This product was dissolved in 0.5 ml of acetic acid and stirred at 55°C for 20 min. The mixture was cooled, ice water added and extracted with ether. The other phase was washed with cold 10% sodium bicarbonate solution, brine, dried over $MgSO_4$, filtered and evaporated to give the expected mixture of 3-acetoxy-1α-hydroxy- and 1α-acetoxy-3-hydroxy isomers, which were separated and purified by HPLC (Zorbax Sil column, 6.4 x 25 cm, 2-propanol in hexane) to give about 70 µg each of compounds VIIa and XIIIa. UV (in EtOH) λ_{max}242.5 (OD 0.72), 251.5 (OD 0.86), 260 (OD 0.57).

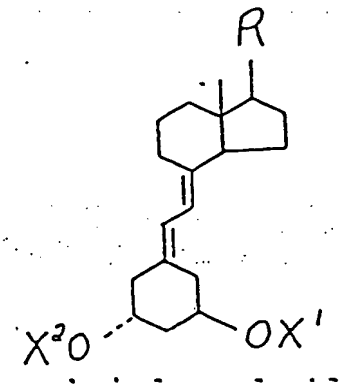
Both 19-nor-1.25-dihydroxyvitamin D_3 acetates VIIa and VIIIa were hydrolyzed in the same manner. Each of the monoacetates was dissolved in 0.5 ml of ether and 0.5 ml 0.1 N KOH in methanol was added. The mixture was stirred under argon atmosphere for 2 h. More ether was added and the organic phase washed with brine, dried over anhydrous $MgSO_4$, filtered and evaporated. The residue was dissolved in a 1:1 mixture of 2-propanol and hexane and passed through a Sep Pak column and washed with the same solvent. The solvents were evaporated and the residue purified by HPLC (Zorbax-Sil, 6.4 x 25 cm, 10% 2-propanol in hexane). The hydrolysis products of VIIa and VIIIa were identical and gave 66 µg of Ia ($X^1=X^2=H$). Mass spectrum (m/z relative intensity) 404 (M^+) (100), 386 (41), 371 (20), 275 (53), 245 (51), 180 (43), 135 (72), 133 (72), 95 (82), 59 (18), exact mass calcd. for $C_{26}H_{44}O_3$ 404.3290, found 404.3272. ^{1}H NMR ($CDCl_3$) δ 0.52 (3H, s, 18-CH_3), 0.92 (3H, d, J=6.9 Hz, 21-CH_3), 1.21 (6H, s, 26-CH_3 and 27-CH_3), 4.02 (1H, m, 3α-H), 4.06 (1H, m, 1β-H), 5.83 (1H, d, J=11.6 Hz, 7-H), 6.29 (IH, d, J=10.7 Hz, 6-H). UV (in EtOH), λ_{max}243 (OD 0.725), 251.5 (OD 0.823), 261 (OD 0.598).

Example 2

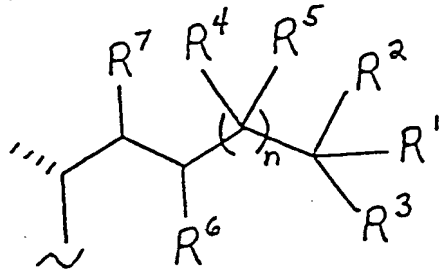
Preparation of 1α-hydroxy-19-nor-vitamin D_3 (Ib)

(a) With vitamin D_3 (IIb) as starting material, and utilizing the conditions of Example 1a, there is obtained known 1α-hydroxy-3,5-cyclovitamin D_3 1-acetate, 6-methyl ether, compound IIIb (X=Ac).

(b) By subjecting intermediate IIIb (X=Ac), as obtained in Example 2a above to the conditions of



where X^1 and X^2 are each independently hydrogen, acyl, alkylsilyl or alkoxyalkyl, and R is alkyl, hydrogen, hydroxyalkyl, fluoroalkyl or a side chain of the formula:



wherein R^1 represents hydrogen, hydroxy or O-acyl, R^2 and R^3 are each independently alkyl, hydroxyalkyl or fluoroalkyl, or, when taken together represent the group -- $(CH_2)_m$ -- where m is an integer from 2 to 5, R^4 is hydrogen, hydroxy, fluorine, O-acyl, alkyl, hydroxyalkyl or fluoroalkyl, R^5 is hydrogen, fluorine, alkyl, hydroxyalkyl or fluoroalkyl, or R^4 and R^5 taken together represent double-bonded oxygen, R^6 and R^7 are each independently hydrogen, hydroxy, O-acyl, fluorine or alkyl, or, R^6 and R^7 taken together form a carbon-carbon double bond, and n is an integer from 1 to 5 and wherein the carbon at any one of positions 20, 22 or 23 in the side chain may be replaced by an O, S, or N atom.

2. A compound according to claim 1 wherein X^1 and X^2 are both hydrogen, R^1 is hydroxy, R^2 and R^3 are each independently methyl, trifluoromethyl, ethyl or propyl, R6 and R^7 are both hydrogen, or together form a carbon-carbon double bond, R^4 and R^5 are both hydrogen and n is 1, 2 or 3.

3. 1α,25-Dihydroxy-19-nor-vitamin D_3.

4. 1α-Hydroxy-19-nor-vitamin D_3.

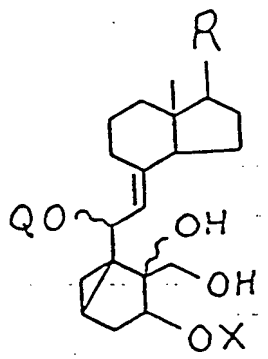
5. 1α,25-Dihydroxy-19-nor-vitamin D_2.

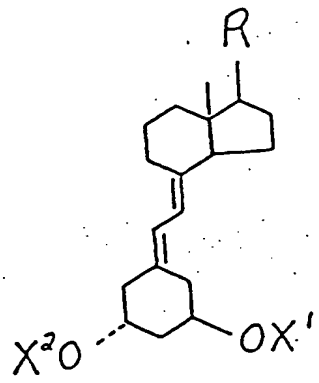
6. 1α-Hydroxy-19-nor-vitamin D_2.

7. 1α-Hydroxy-19-nor-24 epi-vitamin D_2.

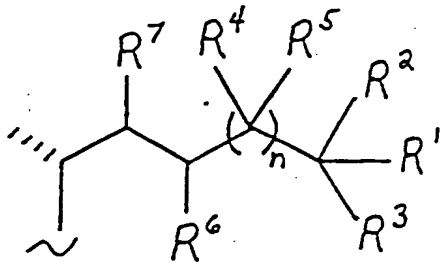
8. 1α,25-Dihydroxy-19-nor-24 epi-vitamin D_2.

9. A compound having the formula:

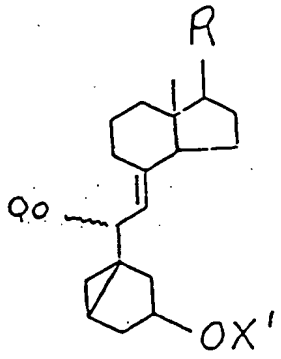




where X^1 and X^2 are each independently hydrogen, acyl, alkylsilyl or alkoxyalkyl, and R is alkyl, hydrogen, hydroxyalkyl, fluoroalkyl or a side chain of the formula:



wherein R^1 represents hydrogen, hydroxy or O-acyl, R^2 and R^3 are each independently alkyl,, hydroxyalkyl or fluoroalkyl, or, when taken together represent the group -- $(CH_2)_m$ -- where m is an integer from 2 to 5, R^4 is hydrogen, hydroxy, fluorine, O-acyl, alkyl, hydroxyalkyl or fluoroalkyl, R^5 is hydrogen, fluorine, alkyl, hydroxyalkyl or fluoroalkyl, or R^4 and R^5 taken together represent double-bonded oxygen, R^6 and R^7 are each independently hydrogen, hydroxy, O-acyl, fluorine or alkyl, or, R^6 and R^7 taken together form a carbon-carbon double bond, and n is an integer from 1 to 5 and wherein the carbon at any one of positions 20, 22 or 23 in the side chain may be replaced by an O, S, or N atom characterised by solvolysing the 1α-hydroxy-10-deoxy cyclovitamin D compound having the formula:



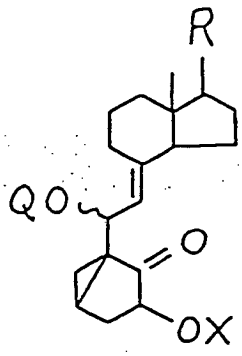
wherein Q is alkyl.

Amended claims in accordance with Rule 86(2) EPC.

1. A compound having the formula:

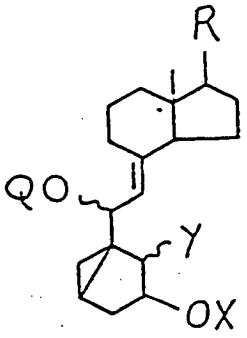
wherein R is as defined in Claim 1, Q represents alkyl and X is hydrogen, acyl, alkylsilyl or alkoxyalkyl.

10. A compound having the formula:



wherein R is as defined in Claim 1, Q represents alkyl and X is hydrogen, acyl, alkylsilyl or alkoxyalkyl.

11. A compound having the formula:



wherein R is as defined in Claim 1, Q represents alkyl, X is hydrogen, acyl, alkylsilyl or alkoxyalkyl, and Y is hydroxy, hydrogen or protected hydroxy where the protecting group is acyl, alkylsilyl or alkoxyalkyl.

12. A pharmaceutical composition which comprises at least one compound as claimed in any one of claims 1 to 8 together with a pharmaceutically acceptable excipient.

13. A composition according to claim 12 wherein the compound is in a solid or liquid vehicle ingestible by, and non-toxic to, mammals.

14. A composition according to claim 12 or 13 wherein the compound is 1α,25-hydroxy-19-nor-vitamin D_3, 1α-hydroxy-19-nor-vitamin D_3, 1α,25-dihydroxy-19-nor-vitamin D_2 or 1α-hydroxy-19-nor-vitamin D_2.

15. A composition according to any one of claims 12 to 14 which contains from 0.5 µg to 50µg of the compound.

16. A composition according to any one of claims 12 to 15 which is suitable for topical administration.

17. A composition according to any one of claims 12 to 15 which is suitable for parenteral administration.

18. A composition according to any one of claims 12 to 15 which is suitable for oral administration.

19. A compound as defined in any one of claims 1 to 8 for inducing cell differentiation in malignant cells.

20. A compound as defined in any one of claims 1 to 8 for inducing cell differentiation in leukemia cells.

21. A compound as defined in any one of claims 1 to 8 for treating a proliferative skin disorder in a mammal.

22. A compound as defined in any one claims 1 to 8 for treating psoriasis.

23. A compound as defined in any one of claims 1 to 8 for treating primary or secondary hyperparathyroidism.

24. A compound as defined in any one of claims 1 to 8 for treating a neoplastic disease.

25. A compound as defined in any one of claims 1 to 8 for use in the treatment of a condition as defined in any one of claims 19 to 24

26. A process for preparing a compound having the formula:

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application number: EP 90 30 2521

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
A	JOURNAL OF THE CHEMICAL SOCIETY PERKIN TRANSACTIONS I, vol. 6, 1978, pages 590-595, London, GB; B. LYTHGOE et al.: "Calciferol and its relatives. Part 22. A direct total synthesis of vitamin D_2 and vitamin D_3[2]" * Page 591, compound 7 *	1	C 07 C 401/00 A 61 K 31/59
A	EP-A-0 250 755 (SUMITOMO PHARMACEUTICALS LTD.) * The whole document *	1,12	
A	WO-A-85 03 300 (WISCONSIN ALUMNI RESEARCH FOUNDATION) * The whole document *	1,9-12,26	
	./.		TECHNICAL FIELDS SEARCHED (Int. Cl.4): C 07 C 401/00 A 61 K 31/00

INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims.

Claims searched completely: 1-18,26

Claims searched incompletely:

Claims not searched: 19-25

Reason for the limitation of the search:

Method for treatment of the human or animal body by surgery or therapy (see Art. 52(4) of the European Patent Convention).

Place of search	Date of completion of the search	Examiner
THE HAGUE	04-05-1990	WATCHORN

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological backgr und
O : non-written disclosure
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T : theory or principle underlying the invention
E : earlier patent document, but published on, r after the filing date
D : document cited in the application
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EPO Form 1503.1 03.82

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int Cl.4)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	WO-A-86 02 649 (WISCONSIN ALUMNI RESEARCH FOUNDATION) * The whole document *	26	
A	US-A-4 448 726 (DE LUCA et al.) * The whole document *	9	TECHNICAL FIELDS SEARCHED (Int Cl.4)